Burosumab in management of X-linked hypophosphataemia: A retrospective cohort study of in growth and serum phosphate levels.

Authors:

EYX Walker[1], TAJ Lindsay[2], J Allgrove[3], M Marlais[1], D Bockenhauer, [1,4] W N Hayes[1,5]

[1] Department of Paediatric Nephrology, Great Ormond Street Hospital, London, UK

[2] Department of Trauma and Orthopaedics, Northwick Park Hospital, London, UK

[3] Department of Paediatric Endocrinology, Great Ormond Street Hospital, London, UK

[4] UCL Department of Renal Medicine, London, UK

[5] UCL Institute of Child Health, London, UK

Address for correspondence:

Dr Wesley Hayes

Department of Nephrology

Great Ormond Street Hospital

Wesley.hayes@gosh.nhs.uk

Abstract: (250 Words Max)

Background:

Burosumab, an anti-fibroblast growth factor 23 monoclonal antibody, improves rickets severity, symptoms and growth in children with X-linked hypophosphataemia (XLH) followed to 64 weeks in clinical trials. International dosing guidance recommends targeting normal serum phosphate concentration, however, some children may not achieve this despite maximal dosing. This study compares clinical outcomes in children with XLH on long-term burosumab treatment who achieved normal phosphate, vs those who did not.

Methods:

Single centre retrospective review of a large paediatric cohort with XLH treated with burosumab. We evaluated growth and biochemical markers of bone health in those who did compared to those who did not achieve normal plasma phosphate concentration.

Results:

Fifty-five children with XLH with median age 11.7 [6.8 – 15.5] years were included. 27 (49%) had low plasma phosphate concentration, and 27 (49%) normal phosphate after a median burosumab treatment duration of 3.3 [IQR 2.6 – 3.7] years. 1 (2%) did not have a recent phosphate level recorded. No difference in growth was found between normal and abnormal phosphate groups ($p = 0.9$). A trend of superior growth in those with normal compared with abnormal alkaline phosphatase level was observed.

Conclusions:

Young children with XLH experience sustained growth on long term burosumab treatment, albeit without normal plasma phosphate concentration in many. Consideration should be made to changing burosumab dosing recommendations to target normalisation of alkaline phosphatase, as opposed to plasma phosphate concentration.

Key messages:

What is already known on this topic:

- Burosumab is a monoclonal antibody that is being used to treat X-linked hypophosphataemia
- Current European guidelines recommend titrating to low-normal values of phosphate

What this study adds:

- Long term data supporting growth if treated with burosumab, irrespective of recent phosphate level

How this study might affect research, practice or policy:

- Treatment guidelines on plasma phosphate targets for burosumab dosing may need to be reconsidered.

Background:

X-linked hypophosphataemia (XLH) is the most common heritable cause of rickets. Girls and boys are affected through X-linked dominant inheritance of loss of function variants in *PHEX*. This leads to dysregulation of Fibroblast Growth Factor 23 (FGF-23) causing kidney tubular phosphate wasting and suppression of 1-alpha hydroxylation of vitamin D[1,2]. Affected children experience growth faltering, severe rickets with bone deformities and pain, and dental complications [1,3,4].

Conventional treatment for X-linked hypophosphataemia comprises oral phosphate and active vitamin D supplementation[5]. Common treatment complications include gastrointestinal symptoms, hypercalcaemia, hypercalciuria and hyperparathyroidism[2].

Burosumab is a recombinant human IgG1 monoclonal antibody that targets FGF-23. Clinical trials in adults and children with X-linked hypophosphataemia demonstrated efficacy of burosumab in increasing serum phosphate levels, improving growth and physical function and reducing pain and the severity of rickets[6–9]. Improved growth in children has been reported to 64 weeks follow up[6–8,10] with one article reporting up to 160 weeks[11]. In 2018, burosumab was authorized by the European Medicines Agency and the US Food and Drug Administration for the treatment of X-linked hypophosphataemia in children with evidence of bone disease aged one year and older [12,13].

International consensus dosing guidance recommends starting burosumab at 0.8mg/kg with subsequent dose titration to achieve fasting serum phosphate

concentration at the lower end of the normal range, with maximum dose 2.0mg/kg (or 90mg) every 2 weeks[14,15].

In a large cohort of young children with XLH treated with burosumab, we observed that plasma phosphate concentration did not reach the normal range in some despite maximal dosing. We hypothesised that normalisation of plasma phosphate may not be the best treatment goal in this group. We therefore compared clinical outcomes for children on long term burosumab treatment with normal vs abnormal plasma phosphate concentration.

Methods:

Study population

We performed a retrospective cohort study of children with X-linked hypophosphataemia treated at our centre between December 2014 and August 2022. Children under 18 years of age with a confirmed diagnosis of X-linked hypophosphataemia undergoing treatment with burosumab were included. Local ethics approval was obtained, and the need for individual consent was waived.

Data collection

Demographic data including age, sex, *PHEX* variant and previous treatment prior to commencing burosumab were collated and then anonymised for analysis.

Clinical anthropometric and biochemical data were extracted at three time points: the commencement of burosumab treatment, approximately one year into treatment and at the patient's most recent clinical review. Data included height (cm), burosumab dose (mg/kg), serum phosphate (PO_4; mmol/l), serum calcium (Ca; mmol/l), parathyroid hormone (PTH; pmol/l), alkaline phosphatase (ALP; IU/l), 25-hydroxy vitamin D (nmol/l) and creatinine (Cr µmol/l), urine phosphate (mmol/l) and urine creatinine (mmol/l). Age and sex specific reference ranges were used throughout. Missing values were recorded as such in the data set. In our clinical practice, we aim to obtain blood tests as close to the next dose as possible, so as to obtain "trough" levels.

Data analysis

Age and sex specific height Z-scores were calculated using the World Health Organization (WHO) AnthroPlus Software tool[16]. To compare phosphate levels for

different age groups, a standardised phosphate value was calculated by dividing the phosphate level by the lower limit of normal for the age group using reference ranges which were generally parsed in 2-3 year intervals[17]. Thus, a value < 1.0 indicates a phosphate level below the lower limit of normal. Similarly, to compare alkaline phosphatase levels, the value was divided by the upper limit of normal for the age/sex group[17]. Thus, a value > 1.0 indicates an ALP level above the upper limit of normal. The reference ranges can be found in supplementary table 1.

Tubular reabsorption of phosphate (TRP, %) and the renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR, mmol/L) were calculated from Payne's equation using urine and serum phosphate and creatinine values[18]. TmP/GFR values were divided by the lower limit of normal for age/sex to give a standardised value.

Continuous data are reported as median [interquartile range], and categorial data as number (%). All statistical analyses were performed using Stata/IC version 15.

Comparison of baseline and most-recent-review anthropometric and biochemical markers

As height measurements were not available for all individuals at baseline and most-recent-review, we assessed within-individual and group aggregate changes in height. Within-individual change was defined as the change in height (cm) and the change in height Z-score between that individual's first and last review. Group-level data was analysed in aggregate and reported as medians [IQR] as above. We used the

Wilcoxon Signed Rank test as a non-parametric, paired, statistical hypothesis test to compare group values at baseline and at the most-recent-review.

Anthropometric and biochemical markers at most-recent-review, stratified by normal or abnormal serum phosphate level

To assess the importance of serum phosphate levels, we stratified the study population by normal or abnormal serum phosphate at the most-recent-review. We then performed a Mann-Whitney U test (unpaired, non-parametric) to check for significant differences between group heights. Values of plasma phosphate were defined as normal if they fell between the age-specific lower and upper limits of normal at the time of the most-recent-review.

Linear regression analysis

To determine whether phosphate level at the most-recent-review was associated with a difference in height Z-score, we performed a series of linear regression analyses. For all models, change in Z-score for height was modelled as the dependent variable, whereas phosphate level (mmol/L) +/- age at the commencement of treatment (years) +/- duration of treatment (years) +/- burosumab dose (mg/kg) were the independent variables. To ensure that the assumptions of linear regression were satisfied, we performed a Shapiro-Wilks test to confirm the normality of the change in Z-score data.

Anthropometric markers at most-recent-review, stratified by normal or abnormal serum alkaline phosphatase levels

To assess the importance of serum alkaline phosphatase (ALP) levels, we also stratified the study population by low/normal or high serum ALP levels at the most-

recent-review. Values of serum ALP were defined as low/normal if they fell below the upper limit of normal (units/Litre) at the time of the most-recent-review. We then performed a Mann-Whitney U test (unpaired, non-parametric) to review the differences between group heights.

Results:

Population demographics

We identified 60 patients with X-linked hypophosphataemia undergoing treatment with burosumab. Of these, 5 patients were excluded as either their initial treatment or current management was elsewhere.

Of the 55 (21 male, 34 female) patients included in the analysis, 52 have confirmed *PHEX* variants with the remaining 3 having positive family histories. Prior to commencing burosumab, 51 were confirmed to be receiving phosphate supplements and 54 were receiving vitamin D.

The median [IQR] age at the commencement of burosumab treatment was 9.0 [3.9 ; 11.0] years, with 17 patients under the age of 5 years. The age at the most recent clinical review was 11.7 [6.8 ; 15.5] years. The median treatment duration with burosumab was 3.3 [2.6 ; 3.7] years. The dose of burosumab at most recent review was 1.17 [0.86 ; 1.41] mg/kg per dose.

Long term change in height:

The age- and sex-adjusted Z-score for height at the commencement of burosumab treatment was -1.19 [-2.32 ; -0.61] or 122.9cm [88.3 ; 138.9] in absolute height. At the most recent clinical review, the Z-score for height was -1.07 [-1.79 ; -0.55] or 141.6cm [115.6 ; 155.4] (figure.1). Of the 50 individuals with data at both time points, the median within-individual change in height Z-score was 0.23 [-0.11 ; 0.51] or 18.2cm [10.0 ; 23.8] in absolute change in height (cm).

Differences in biochemistry

A comparison of group-level biochemical data at the onset of treatment and most-recent-review demonstrated several statistically significant differences. Serum phosphate levels significantly increased (baseline: 0.81 mmol/l; review: 1.06 mmol/l; $p < 0.01$). Serum alkaline phosphatase levels significantly decreased (392U/L; 209U/L; $p < 0.01$), as were serum calcium levels (2.33 mmol/L; 2.30 mmol/L; $p < 0.01$).

Total 25-hydroxy-vitamin D levels were higher at the most-recent-review (68 nmol/l; 88 nmol/l; $p= 0.06$), though 31 of 55 were concomitantly prescribed vitamin D. Tubular reabsorption of phosphate (83% ; 94%) and the renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR, 0.57 mmol/L ; 1.01 mmol/L) were significantly higher ($p < 0.01$) at most recent review.

Comparison of parameters stratified by phosphate level at most-recent-review

At most recent review, 27 patients (49%) had normal phosphate levels, 27 patients (49%) remained hypophosphataemic and 1 did not have a recorded value. This division between those with normal phosphate levels and those that were hypophosphataemic was similar at the 1-year review (figure 2), indicating that the most recent review was a valid time point for group stratification.

At baseline, these two groups were not significantly different across anthropometric and biochemical measures, except for age, with the normal-phosphate group older by 2.6 years ($p = 0.01$). All other baseline characteristics were similar; these results are presented in Table 1.

The change in height Z-score was not statistically significant, nor was the duration of treatment; however, the dose of burosumab was higher in the hypophosphataemic group. A further comparison of various anthropometric and biochemical markers is demonstrated in Figure 3 with the reference ranges for normal delineated with the black line.

Age at the commencement of treatment of burosumab

The age of commencement of burosumab treatment was negatively associated with height gain (Figure 4). Linear regression analysis was performed, and this association remained significant ($p < 0.01$) after adjustment for phosphate level at most-recent-review, duration of burosumab treatment and dose of burosumab at most recent review. By contrast, phosphate level, duration of treatment or burosumab dose were not significantly associated with change in height in any model.

Comparison of parameters stratified by ALP level at most-recent-review

41 patients recorded normal/low serum ALP levels at the most-recent-review with a median ALP level of 200 [161 ; 273] IU/L, or, as a proportion of the upper limit of normal, 0.53 [0.45 ; 0.74]. There was a median change in height Z-score of 0.28 [-0.09 ; 0.51]. 11 patients recorded higher than normal ALP levels with a median value of 260 [190 ; 388] IU/L, or as a proportion of the upper limit of normal, 1.51 [1.12 ; 2.90], with a change in height Z-score of 0.02 [-0.16 ; 0.07] (see Figure 5). This was not statistically significant ($p = 0.56$).

Discussion:

Treatment of X-linked hypophosphataemia with the FGF-23 monoclonal antibody, burosumab, has been shown to improve phosphate metabolism, decrease severity of rickets and improve growth and activity, as well as reduce pain[6]. Due to the relatively recent availability of burosumab, current published experience is mostly limited to short follow-up periods (up to 64 weeks) [6–8,10] with only one article recently published up to 160 weeks[11]. This study shows longer-term data supporting a significant albeit modest change in height in children treated with regular (every 2 weeks) burosumab. Furthermore, growth did not differ between those who achieved normal plasma phosphate levels on burosumab treatment compared to those who did not. Of note, prior to the initiation of burosumab, the median height was higher and alkaline phosphatase lower than in previous studies, which may reflect better baseline disease control[6,7]. This may have resulted in a relatively less marked clinical effect of Burosumab and thereby impact the generalisability of the current study results.

Some of our data is in keeping with previous literature: no patient had a serum phosphate level above the upper limit of the normal range; nor was there a notable change in serum calcium levels or serum parathyroid hormone levels [6]. There was a statistically significant decrease in serum alkaline phosphatase level. While there was a trend for better growth in those with normal/low alkaline phosphatase levels at most recent review, the small number in the higher-than-normal level group precluded definitive analysis. These differences could be explained by variations in pubertal timing or status by which a higher growth rate could be linked with lower ALP, so further studies could consider reviewing this. The finding of higher PTH activity and lower tubular reabsorption of phosphate in the normal phosphate group was surprising,

given that PTH is a phosphaturic hormone. The underlying cause of this cannot be determined from our data. Still, it may relate to less enteral phosphate absorption or a higher degree of bone deposition in the low phosphate group. However, since the median PTH was in the normal range in both groups, it is difficult to ascribe much physiological relevance to this difference.

European guidelines suggest targeting fasting serum levels of phosphate within the lower end of the normal reference range for age[15]. In contrast, our data suggest that approximately half of our patients are currently not achieving normal phosphate levels, but despite this they are still achieving good growth and biochemical parameters that are similar to those achieving normal phosphate levels and consistent with previous studies. This finding is in keeping with a previous study which demonstrated good growth on conventional treatment despite persistent hypophosphataemia[1]. These data call into question whether normal phosphate levels are an appropriate treatment goal[15].

Burosumab is an expensive drug and is administered subcutaneously, with many patients requiring multiple vials per dose and there are subsequent, though largely well-tolerated adverse effects such as injection-site reactions. Those with lower phosphate levels were receiving higher doses of burosumab per kilogram of bodyweight. Our data question the clinical need for such higher dosing based on phosphate levels and may prompt reconsideration of current guidelines.

Early treatment with conventional therapy for children with X-linked hypophosphataemia is associated with improved growth and skeletal outcomes[1,19]. Burosumab improves outcomes both for the younger and older children with X-linked

hypophosphataemia[19], but our current data support the importance of early treatment with burosumab to maximise height gain for children with XLH.

This retrospective cohort study has some limitations. Where values were age- and/or sex-specific, values were divided by the upper/lower limits of normal to compare groups. Z-scores would be an alternative method; however, we did not have the distribution nor standard deviations to accurately calculate the value. Data were not complete for all patients as some clinical reviews coincided with the covid-19 pandemic when outpatient face-to-face clinical interactions were reduced. Furthermore, regular x-rays and Thacher rickets scores were not performed, nor were data collected for dental complications, which would add to the previous literature. Our treatment strategy was guided by European guidelines to target normal serum phosphate concentration. Nevertheless, confounding cannot be excluded given the retrospective real-world study design. However, this is a large cohort of patients with clinically significant findings which challenge the current recommendations.

Conclusions:

The introduction of burosumab, the anti-FGF-23 monoclonal antibody for treatment of X-linked hypophosphataemia has benefitted children through improving phosphate homeostasis and decreasing the severity of rickets[6]. This study demonstrates sustained growth with burosumab treatment in a real-world cohort of young children.

The data confirm that age of the commencement of burosumab is important to optimise growth. Newborns and infants of affected families or those with suspected X-linked hypophosphataemia should be screened early to avoid delays in starting treatment.

The majority of children in this study did not achieve normal phosphate levels on burosumab with no apparent detriment to growth. These data call into question current recommendations to target low-normal serum phosphate levels as a treatment goal. The data suggest that normalisation of serum alkaline phosphatase may be a more clinically relevant goal for burosumab dose titration, but larger prospective studies are needed for confirmation.

Disclosures: all authors have no conflicts of interest.

References:

1 Quinlan C, Guegan K, Offiah A, Neill RO, Hiorns MP, Ellard S *et al.* Growth in PHEX-associated X-linked hypophosphatemic rickets: the importance of early treatment. *Pediatr Nephrol* 2012; 27: 581–588.

2 Carpenter TO, Imel EA, Holm IA, Beur SMJ de, Insogna KL. A clinician's guide to X-linked hypophosphatemia. *J Bone Miner Res* 2011; 26: 1381–1388.

3 Padidela R, Nilsson O, Makitie O, Beck-Nielsen S, Ariceta G, Schnabel D *et al.* The international X-linked hypophosphataemia (XLH) registry (NCT03193476): rationale for and description of an international, observational study. *Orphanet J Rare Dis* 2020; 15: 172.

4 Skrinar A, Dvorak-Ewell M, Evins A, Macica C, Linglart A, Imel EA *et al.* The Lifelong Impact of X-Linked Hypophosphatemia: Results From a Burden of Disease Survey. *J Endocr Soc* 2019; 3: 1321–1334.

5 Glorieux FH, Marie PJ, Pettifor JM, Delvin EE. Bone Response to Phosphate Salts, Ergocalciferol, and Calcitriol in Hypophosphatemic Vitamin D-Resistant Rickets. *New Engl J Medicine* 1980; 303: 1023–1031.

6 Carpenter TO, Whyte MP, Imel EA, Boot AM, Högler W, Linglart A *et al.* Burosumab Therapy in Children with X-Linked Hypophosphatemia. *New Engl J Med* 2018; 378: 1987–1998.

7 Imel EA, Glorieux FH, Whyte MP, Munns CF, Ward LM, Nilsson O *et al.* Burosumab versus conventional therapy in children with X-linked hypophosphataemia: a randomised, active-controlled, open-label, phase 3 trial. *Lancet* 2019; 393: 2416–2427.

8 Whyte MP, Carpenter TO, Gottesman GS, Mao M, Skrinar A, Martin JS *et al.* Efficacy and safety of burosumab in children aged 1–4 years with X-linked hypophosphataemia: a multicentre, open-label, phase 2 trial. *Lancet Diabetes Endocrinol* 2019; 7: 189–199.

9 Schindeler A, Biggin A, Munns CF. Clinical Evidence for the Benefits of Burosumab Therapy for X-Linked Hypophosphatemia (XLH) and Other Conditions in Adults and Children. *Front Endocrinol* 2020; 11: 338.

10 Ramos SM, Gil-Calvo M, Roldán V, Martínez AC, Santos F. Positive Response to One-Year Treatment With Burosumab in Pediatric Patients With X-Linked Hypophosphatemia. *Frontiers Pediatrics* 2020; 8: 48.

11 Linglart A, Imel EA, Whyte MP, Portale AA, Högler W, Boot AM *et al.* Sustained Efficacy and Safety of Burosumab, a Monoclonal Antibody to FGF23, in Children With X-Linked Hypophosphatemia. *J Clin Endocrinol Metabolism* 2021; 107: 813–824.

12 European Medicines Agency. Crysvita EPAR product information. Summary of product characteristics. https://www.ema.europa.eu/en/documents/product-information/crysvita-epar-product-information_en.pdf (accessed 31 Jul2022).

13 US Food and Drug Administration. Crysvita (prescribing information). https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/761068s000lbl.pdf (accessed 31 Jul2022).

14 Haffner D, Emma F, Eastwood DM, Duplan MB, Bacchetta J, Schnabel D *et al.* Clinical practice recommendations for the diagnosis and management of X-linked hypophosphataemia. *Nat Rev Nephrol* 2019; 15: 435–455.

15 Trombetti A, Al-Daghri N, Brandi ML, Cannata-Andía JB, Cavalier E, Chandran M *et al.* Interdisciplinary management of FGF23-related phosphate wasting syndromes: a Consensus Statement on the evaluation, diagnosis and care of patients with X-linked hypophosphataemia. *Nat Rev Endocrinol* 2022; 18: 366–384.

16 World Health Organization Anthro Survey Analyser and other tools. https://www.who.int/tools/child-growth-standards/software (accessed 31 Jul2022).

17 Lockitch G, Halstead AC, Albersheim S, MacCallum C, Quigley G. Age- and sex-specific pediatric reference intervals for biochemistry analytes as measured with the Ektachem-700 analyzer. *Clin Chem* 1988; 34: 1622–1625.

18 Payne RB. Renal Tubular Reabsorption of Phosphate (TmP/GFR): Indications and Interpretation. *Ann Clin Biochem* 1997; 35: 201–206.

19 Ward LM, Glorieux FH, Whyte MP, Munns CF, Portale AA, Högler W *et al.* Effect of Burosumab Compared With Conventional Therapy on Younger vs Older Children With X-linked Hypophosphatemia. *J Clin Endocrinol Metabolism* 2022; 107: e3241–e3253.

404 Table Legend:

405 Table 1. A comparison of anthropometric and biochemical markers at baseline and

406 most recent clinical review based on most recent phosphate level.

407

Figure Legends:

Figure 1. A box plot demonstrating height Z-scores at commencement of treatment, 1-year of treatment, and most recent review. Height Z-score variance decreased with duration of treatment, with a trend towards more normal heights.

Figure 2. A box plot demonstrating serum phosphate level as a proportion of lower limit of normal (age-adjusted) at commencement of treatment, 1-year of treatment, and at the most recent review. The plots demonstrate a tendency towards normalised phosphate levels with treatment.

Figure 3. Box plots of growth and biochemical markers at most recent review stratified by phosphate level. The results of a test of significance for these comparisons can be found in Table 1.

Figure 4. The relationship between height Z-score and age at first dose of Burosumab (years). The scatter plot shows that greater change in height Z-score is associated with commencing Burosumab treatment at an earlier age.

Figure 5. A two-way scatter plot of change in height Z-score against age at first dose (years), stratified by normal/abnormal serum phosphate at most recent review (left panel) and normal/high serum alkaline phosphatase level at most recent review (right panel). The left panel demonstrates that there is no difference in change in height Z-score between those with normal or abnormal serum phosphate levels. However, starting Burosumab earlier improves change in height Z-score. The right panel shows that there is no difference between change in height Z-scores and ALP

433 levels. However, this figure shows a trend that a normal ALP level is correlated with
434 improved change in height Z-score.
435
436